MH 17 T.

October 29, 1943.

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Dear Jacques:

Some time ago I read, with great attention and interest, the two papers in the May J. Immunol. by your group and put them aside for further study. An enforced day in bed gave me the opportunity yesterday, and goaded by your challenge beginning at the bottom of p.288, I made a few calculations, with the following result:

First, let me say that the "contrary finding...with anti-Ea" is not an isolated result, as you infer: it was equally definitely shown with Kabat (J. Exp. Med., 1937, 66, 229, esp. Table V) in the horse serum albumin-rabbit anti-HSA system, and at the S.A.B. meeting in 1940 (J.Bact. 39,37) Mayer and I told of the presence of 10% or more "univalent" antibody in other systems. Now, to add to this, we have your own data, which clearly show the inhomogeneity of rabbit antiphage, unless I, too, have entirely misinterpreted them, as I believe you have.

Taking as a basis the antibody content of antiserum 29 as 0.6 mg. A N per ml. (Table 2 and p.271 of Paper 1) and the statement on p. 288 that about 40% A had been removed from 29A, let us get to work on the pertinent portion of Table 2 on p. 285:

	.0125 ml 29 contains 7.5 γA			0.0167 ml 29A contains 6.0 γA		
	Ser P	% absd.	γA N absd.	Ser P	% absd.	γA N absd.
0						
1	(320)	(ca.10?)	(ca.1)	400*	25†	1.5
2	160	21	1.5	200*	37†	2.3
3.	80	49	3.7	100*	57†	3.5

etc., with the results naturally approaching each other as one nears 100%.

* Ratio at which A content = that in corresponding tube of 29.
† By rough extrapolation.

At equal A content, the only point at which comparisons are permissible, it seems quite clear that whole anti-phage has been very different combining ratios from anti-P with the "cream" skimmed off. You will note, too, that this comparison is made in the region of A excess, where we also made ours. Comparisons at the 50% P neutralization end are not competent to decide this point, as you seem to think (Fig. 1, etc.), for in the antigen-excess region A/antigen ratios are much lower, and even with quantitative N estimations in error by only a very few % it is difficult to decide between, let us say, 3.3 and 3.0. Another very clear demonstration of the inhomogeneity of A (which you use to show just the opposite) is also given in your Table 4. Here, I take it, if you use Fig. 1, although this has to be dug out of the text and is not stated as ordinate, " expected neutrn" and "obsd. neutrn." refer to P, not A. If this is correct, these two columns are crucial, for as a rule the observed values are notably higher than the calculated, as I could have predicted from our work. As you see, the lower-ratio A combines with more P per ℊ than does "whole" A.

Again, it seems to me the values of k in Table 3 of Paper 1 also clearly show the difference between whole and partly absorbed 29 for if k is a function of the A content, as you say, and doubles in the case of Serum 1 when P_o goes from 10^6 to 10^9 the two k's for 29 and 29A should be about equal. However, they are nearly as 2: 1!

Taking up another point, the size of P, which may apparently vary from the intriguing forms shown in the electron microscope down to 50,000 or so, why need one stop at 27 lytic units? Stokinger and I showed (J. Exp. Med., 37:66, 73) that in the region of A excess with thyroglobulin of M.W.750,000 T_2A_{40} was easily possible. T_2 is apparently a disc (Svedberg said "a pancake"), so that it requires little imagination to stick 40A on to it, especially end on. T_2 also dissociates on dilution. If P is not spherical and is 'way up in the millions, why not 4630A? That is only 100 times T_2A_{40}, and would only make P = 100 x 10^6.

However, I am not very much at home with 'phage and found myself unable to be as sure about P as about A. However, since you were also very sure about A, I'm still a little worried about some of these calculations regarding P as well. You see, as I wrote to Hershey, I never could subscribe to that third precipitin reaction paper, and what you've done in this one in following out the idea of antibody homogeneity makes it still more apparent that the evidence lies all the other way.

However, it's possible that my arithmetic is bad, so I'll be much interested in your come-back. More power to you if you can drive it home! And because I don't agree with you on A, don't think that I'm not filled with admiration at the beautiful new technical methods you've introduced and the accuracy and reproducibility with

which you now can handle 'phage!

With cordial greetings to you all,

Sincerely

Michael Heidelberger

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P.S. My former student and collaborator, Manfred Mayer, makes the following additional observations:

The necessity for the parallelism of the curves in Fig. 1 if the sera differ only in A content should be proved, not stated, since the exact function connecting A and neutralization is not known. For example, equations such as we use indicate the A content of 2 different sera to vary as the squares of the P concentrations giving similar % neutralization.

M. H.